CS681: Advanced Topics in Computational Biology

Week 4, Lectures 1-2-3

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Read Mapping

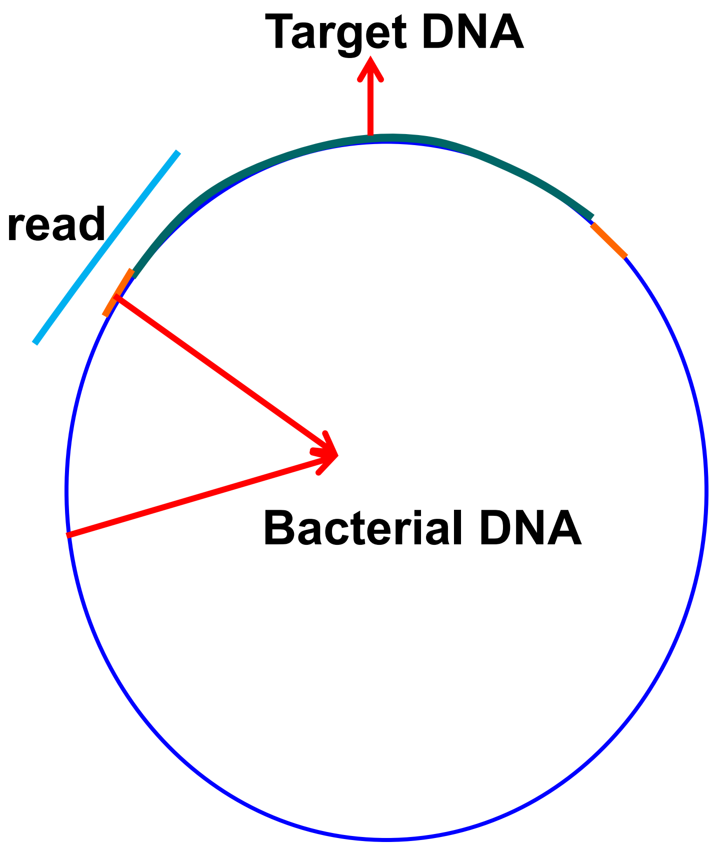
- When we have a reference genome & reads from DNA sequencing, which part of the genome does it come from?
- Challenges:
  - Sanger sequencing
    - Cloning vectors
    - Millions of long (~1000 bp reads)
  - Next-Gen sequencing:
    - Billions of short reads
  - Common: sequencing errors
    - More prevalent in NGS
  - Common: contamination
    - Typically ~2-3% of reads come from different sources; i.e. human resequencing contaminated with yeast, E. coli, etc.
  - Common: Repeats & Duplications

Read Mapping

- Accuracy
  - Due to repeats, we need a confidence score in alignment
- Sensitivity
  - Don’t lose information
- Speed!!!!!!!
- Think of the memory usage
- Output
  - Keep all needed information, but don’t overflow your disks
- All read mapping algorithms perform alignment at some point (read vs. reference)

Sanger vs NGS: cloning vectors

- Sanger reads may contain sequence from the cloning vector; thus mapping needs *local alignment.*
- No cloning vectors in NGS, *global alignment* is fine.



Local vs. Global Alignment

- The <u>Global Alignment Problem</u> tries to find the best alignment from **start** to **end** for two sequences
- The <u>Local Alignment Problem</u> tries to find the subsequences of two sequences that give the best alignment
- Solutions to both are extensions of Longest Common Subsequence

Local vs. Global Alignment (cont’d)

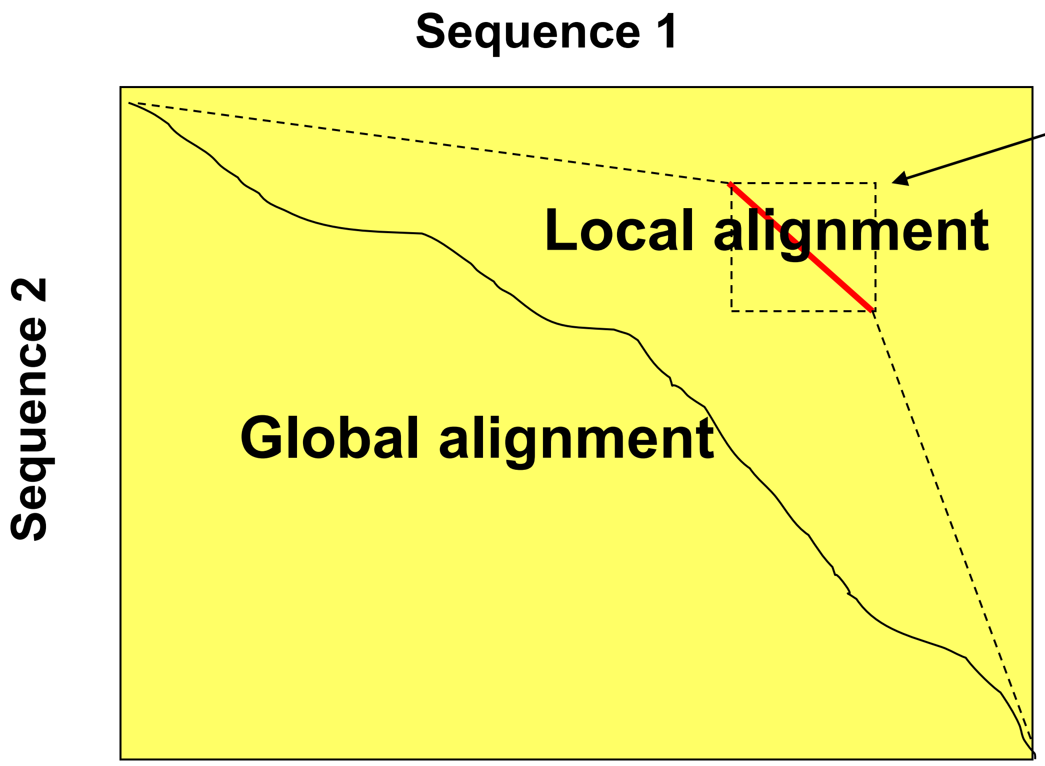
- **Global Alignment**

```
--T—-CC-C-AGT—-TATGT-CAGGGGACACG—A-GCATGCAGA-GAC
  |  || |  ||  | | | |||    || | | |  | ||||   |
AATTGCCGCC-GTCGT-T-TTCAG----CA-GTTATG—T-CAGAT--C
```

- **Local Alignment—better alignment to find conserved segment**

```
                  tccCAGTTATGTCAGgggacacgagcatgcagagac
                     ||||||||||||
aattgccgccgtcgttttcagCAGTTATGTCAGatc
```

Local Alignment: Example



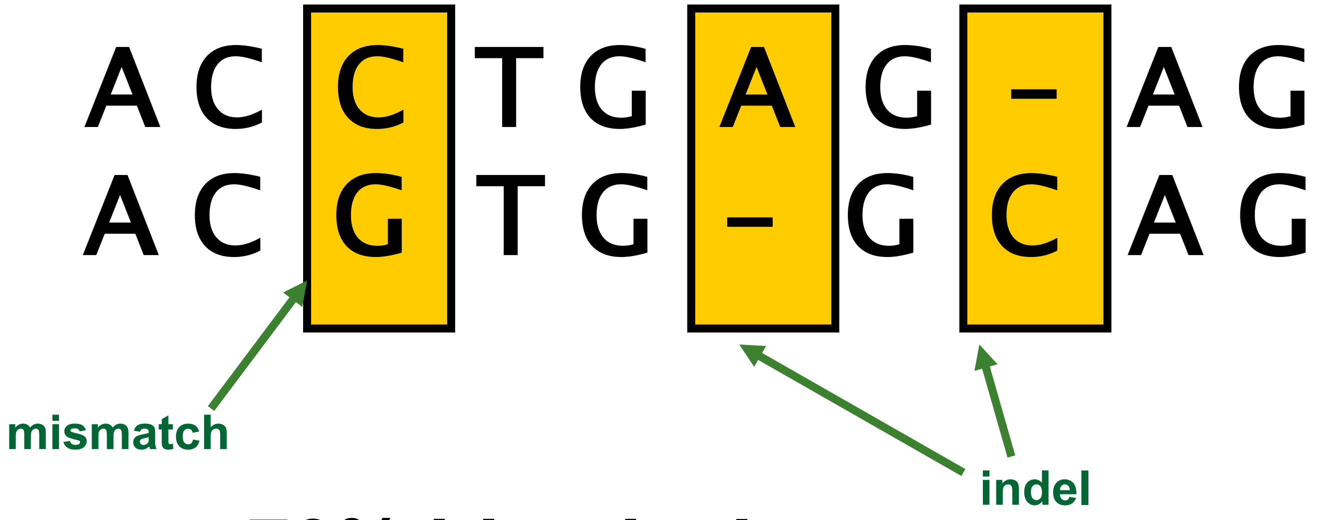
Compute a “mini” Global Alignment to get Local

Measuring Similarity

- Measuring the extent of similarity between two sequences
  - Based on percent sequence identity
  - Based on conservation

Percent Sequence Identity

- The extent to which two nucleotide or amino acid sequences are invariant



70% identical

Global Alignment

- Hamming distance:
  - Easiest; two sequences s_1, s_2, where $|s_1|=|s_2|$
  - $HD(s_1, s_2)$ = #mismatches
- Edit distance
  - Include indels in alignment
  - Levenstein's edit distance algorithm, simple recursion with match score = +1, mismatch=indel=-1; O(mn)
  - Needleman-Wunsch: extension with scoring matrices and *affine gap penalties;* O(mn)

Edit Distance vs Hamming Distance

Hamming distance always compares i-th letter of v with i-th letter of w

```
v  = ATATATAT
     ||||||||
w  = TATATATA
```

Hamming distance:

d(v, w)=8

Edit distance may compare i-th letter of v with j-th letter of w

```
v  =  -ATATATAT
        |||||||
w  =  TATATATA -
```

Edit distance:

d(v, w)=2

(one insertion and one deletion)

The Global Alignment Problem

Find the best alignment between two strings under a given scoring schema

Input : Strings **v** and **w** and a scoring schema
Output : Alignment of maximum score

$\uparrow\rightarrow = -б$
$= 1$ if match
$= -\mu$ if mismatch

$$s_{i,j} = \max \begin{cases} s_{i-1,j-1} + 1 & \text{if } v_i = w_j \\ s_{i-1,j-1} - \mu & \text{if } v_i \neq w_j \\ s_{i-1,j} - \sigma \\ s_{i,j-1} - \sigma \end{cases}$$

***μ* : mismatch penalty**
***σ* : indel penalty**

Scoring matrices

- Different scores for different character match & mismatches
- Amino acid substitution matrices
  - PAM
  - BLOSUM
- DNA substitution matrices
  - DNA is less conserved than protein sequences
  - Less effective to compare coding regions at nucleotide level

Scoring Matrices

To generalize scoring, consider a (4+1) x(4+1) **scoring matrix** δ.

In the case of an amino acid sequence alignment, the scoring matrix would be a (20+1)x(20+1) size. The addition of 1 is to include the score for comparison of a gap character "-".

This will simplify the algorithm as follows:

$$s_{i,j} = max \begin{cases} s_{i-1,j-1} + \delta(v_i, w_j) \\ s_{i-1,j} + \delta(v_i, -) \\ s_{i,j-1} + \delta(-, w_j) \end{cases}$$

Scoring Indels: Naive Approach

- A fixed penalty σ is given to every indel:
  - $-\sigma$ for 1 indel,
  - -2σ for 2 consecutive indels
  - -3σ for 3 consecutive indels, etc.

Can be too severe penalty for a series of 100 consecutive indels

Affine Gap Penalties

- In nature, a series of *k* indels often come as a single event rather than a series of *k* single nucleotide events:

```
ATA__GC        ATAG_GC
ATATTGC        AT_GTGC
```

This is more likely.

Normal scoring would give the same score for both alignments

This is less likely.

Accounting for Gaps

- *Gaps*- contiguous sequence of spaces in one of the rows
- Score for a gap of length x is:

  $$-(\rho + \sigma x)$$

  where ρ >0 is the penalty for introducing a gap:

  gap opening penalty

  ρ will be large relative to σ*:*

  gap extension penalty

  because you do not want to add too much of a penalty for extending the gap.

Affine Gap Penalties

- Gap penalties:
  - $-\rho-\sigma$ when there is 1 indel
  - $-\rho-2\sigma$ when there are 2 indels
  - $-\rho-3\sigma$ when there are 3 indels, etc.
  - $-\rho- x\cdot\sigma$ (-gap opening - x gap extensions)
- Somehow reduced penalties (as compared to naïve scoring) are given to runs of horizontal and vertical edges

Affine Gap Penalty Recurrences

$$\overset{\downarrow}{s}_{i,j} = \max \begin{cases} \overset{\downarrow}{s}_{i-1,j} - \sigma & \text{Continue Gap in } w \text{ (deletion)} \\ s_{i-1,j} - (\rho+\sigma) & \text{Start Gap in } w \text{ (deletion): from middle} \end{cases}$$

$$\overrightarrow{s}_{i,j} = \max \begin{cases} \overrightarrow{s}_{i,j-1} - \sigma & \text{Continue Gap in } v \text{ (insertion)} \\ s_{i,j-1} - (\rho+\sigma) & \text{Start Gap in } v \text{ (insertion):from middle} \end{cases}$$

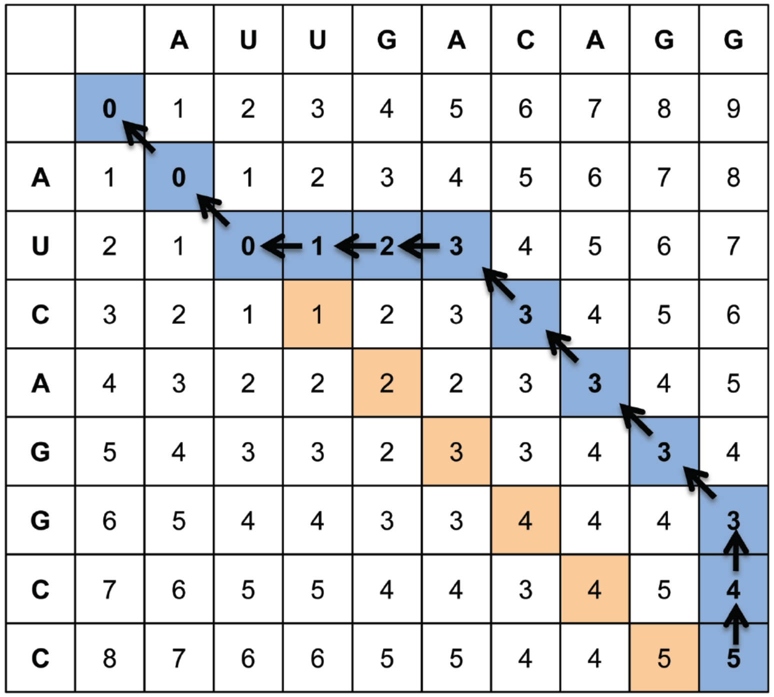
$$s_{i,j} = \max \begin{cases} s_{i-1,j-1} + \delta(v_i, w_j) & \text{Match or Mismatch} \\ \overset{\downarrow}{s}_{i,j} & \text{End deletion: from top} \\ \overrightarrow{s}_{i,j} & \text{End insertion: from bottom} \end{cases}$$

Ukkonen's Approximate String Matching

Regular alignment

Observation:
If max allowed edit distance is small, you don't go far away from the diagonal

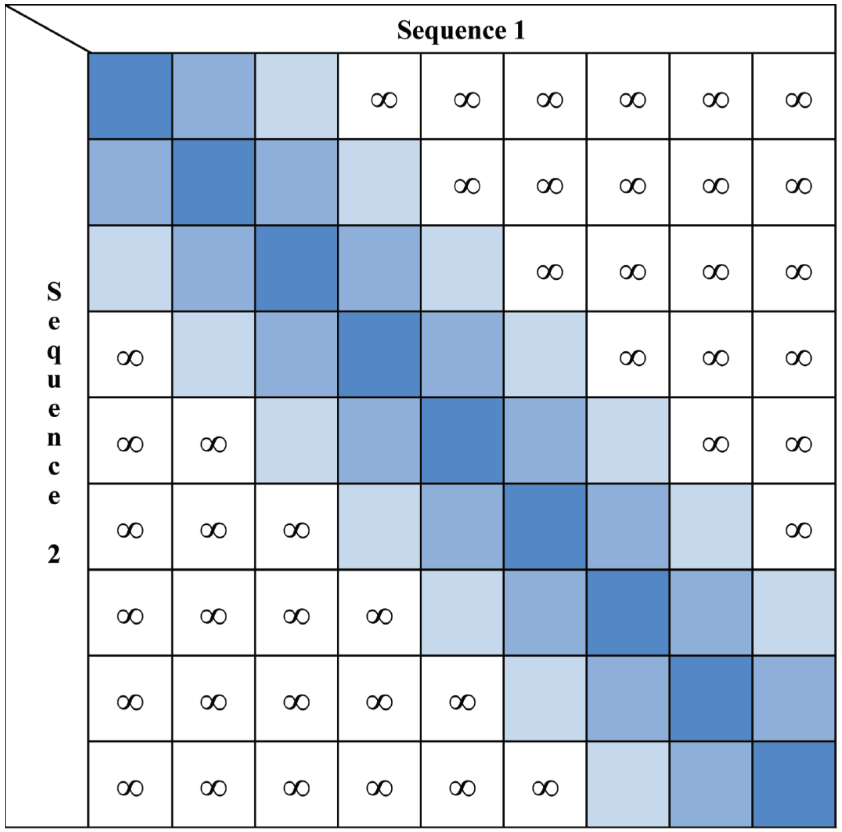
(global alignment only)



		A	U	U	G	A	C	A	G	G
	0	1	2	3	4	5	6	7	8	9
A	1	**0**	1	2	3	4	5	6	7	8
U	2	1	**0**	**1**	**2**	**3**	4	5	6	7
C	3	2	1	1	2	3	**3**	4	5	6
A	4	3	2	2	2	2	3	**3**	4	5
G	5	4	3	3	2	3	3	4	**3**	4
G	6	5	4	4	3	3	4	4	4	**3**
C	7	6	5	5	4	4	3	4	5	**4**
C	8	7	6	6	5	5	4	4	5	**5**

AUUGACAGG - -
AU - - - CAGGCC

Ukkonen’s alignment



If maximum allowed number of indels is *t*, then you only need to calculate 2*t*-1 diagonals around the main diagonal.

The Local Alignment Recurrence

- The largest value of $s_{i,j}$ over the whole edit graph is the score of the best local alignment.

- The recurrence:

$$s_{i,j} = \max \begin{cases} 0 \\ s_{i-1,j-1} + \delta(v_i, w_j) \\ s_{i-1,j} + \delta(v_i, -) \\ s_{i,j-1} + \delta(-, w_j) \end{cases}$$

there is only this change from the original recurrence of a Global Alignment - since there is only one “free ride” edge entering into every vertex

Smith-Waterman Algorithm

Smith-Waterman

$$s_{i,j} = \max \begin{cases} 0 \\ s_{i-1,j-1} + \delta(v_i, w_j) \\ s_{i-1,j} + \delta(v_i, -) \\ s_{i,j-1} + \delta(-, w_j) \end{cases}$$

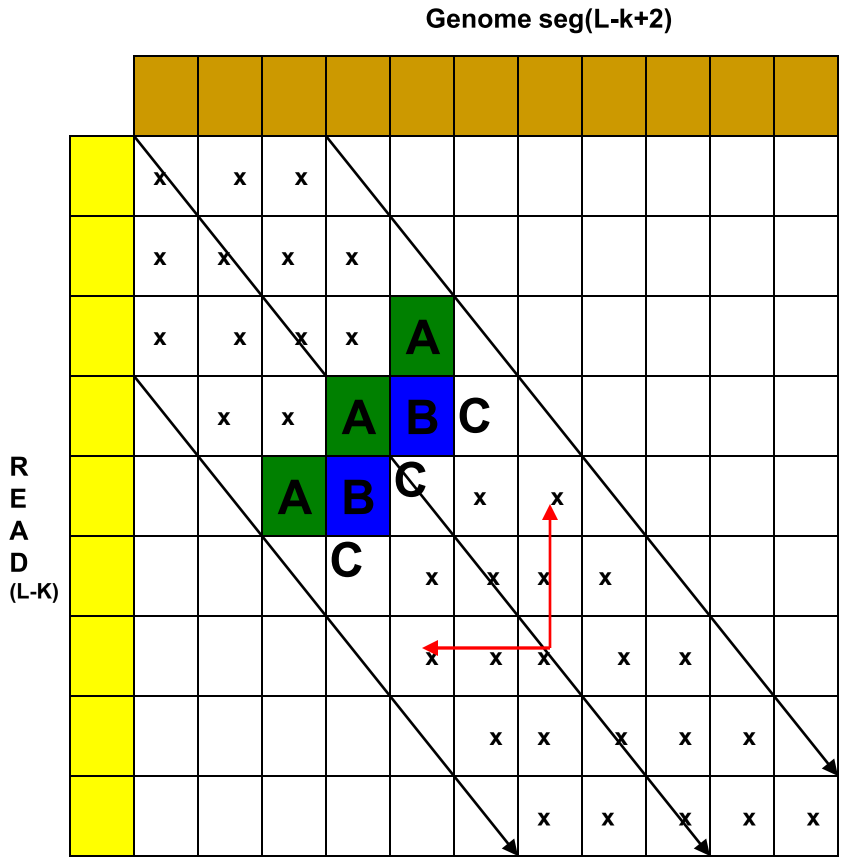
- Start from the maximum score s(i,j) on the alignment matrix
- Move to m(i-1, j), m(i, j-1) or m(i-1, j-1) until s(i,j)=0 or i=j=0
- O(mn)

Faster Implementations

- GPGPU: general purpose graphics processing units
  - Should avoid branch statements (if-then-else)
- FPGA: field programmable gate arrays
- SIMD instructions: single-instruction multiple data
  - SSE instruction set (Intel)
    - Also available on AMD processors
    - Same instruction is executed on multiple data concurrently

Alignment with SSE

- Applicable to both global and local alignment
- Using SSE instruction set we can compute each diagonal in parallel
- Each diagonal will be in saved in a 128 bit SSE specific register
- The diagonal C, can be computed from diagonal A and B in parallel
- Number of SSE registers is limited, we can not hold the matrix, but only the two last diagonals is needed anyway.



READ MAPPERS

Mapping Reads

Problem: We are given a read, *R,* and a reference sequence, *S*. Find the best or all occurrences of *R* in *S*.

Example:

R = AAACGAGTTA

S = TTAATGC*AAACGAGTTA*CCCAATATATAT*AAACCAGTTA*TT

Considering no error: one occurrence.

Considering up to 1 substitution error: two occurrences.

Considering up to 10 substitution errors: many meaningless occurrences!

Don't forget to search in both forward and reverse strands!!!

Mapping Reads (continued)

Variations:

- Sequencing error
  - No error: R is a perfect subsequence of S.
  - Only substitution error: R is a subsequence of S up to a few substitutions.
  - Indel and substitution error: R is a subsequence of S up to a few short indels and substitutions.
- Junctions (for instance in alternative splicing)
  - Fixed order/orientation

    $R = R_1R_2\ldots R_n$ and R_i map to different non-overlapping loci in S, but to the same strand and preserving the order.
  - Arbitrary order/orientation

    $R = R_1R_2\ldots R_n$ and R_i map to different non-overlapping loci in S.

Mapping algorithms

- Two main “styles”:
  - Hash based seed-and-extend (hash table, suffix array, suffix tree)
    - Index the k-mers in the genome
      - Continuous seeds and gapped seeds
    - When searching a read, find the location of a k-mer in the read; then extend through alignment
    - Requires large memory; this can be reduced with cost to run time
    - More sensitive, but slow
  - Burrows-Wheeler Transform & Ferragina-Manzini Index based aligners
    - BWT is a data compression method used to compress the genome index
    - Perfect hits can be found very quickly, memory lookup costs increase for imperfect hits
    - Reduced sensitivity

“Long” read mappers

- BLAST, MegaBLAST, BLAT, LASTZ can be used for Sanger, 454, Ion Torrent
  - Hash based
  - Extension step is done using Smith-Waterman algorithm
  - BLAST and MegaBLAST have additional scoring scheme to order hits and assign confidence values
  - 454/Ion Torrent only: PASH, Newbler

Short read mappers

- Hash based
  - Illumina: mrFAST, mrsFAST, MAQ, MOSAIK, SOAP, SHRiMP, etc.
    - MOSAIK requires ~30GB memory
    - Others limit memory usage by dividing genome into chunks
    - mrFAST, SHRiMP have SSE-based implementation
    - MAQ: Hamming distance only
  - SOLiD: drFAST, BFAST, SHRiMP, mapreads
  - GPGPU implementations: Saruman, Mummer-GPU

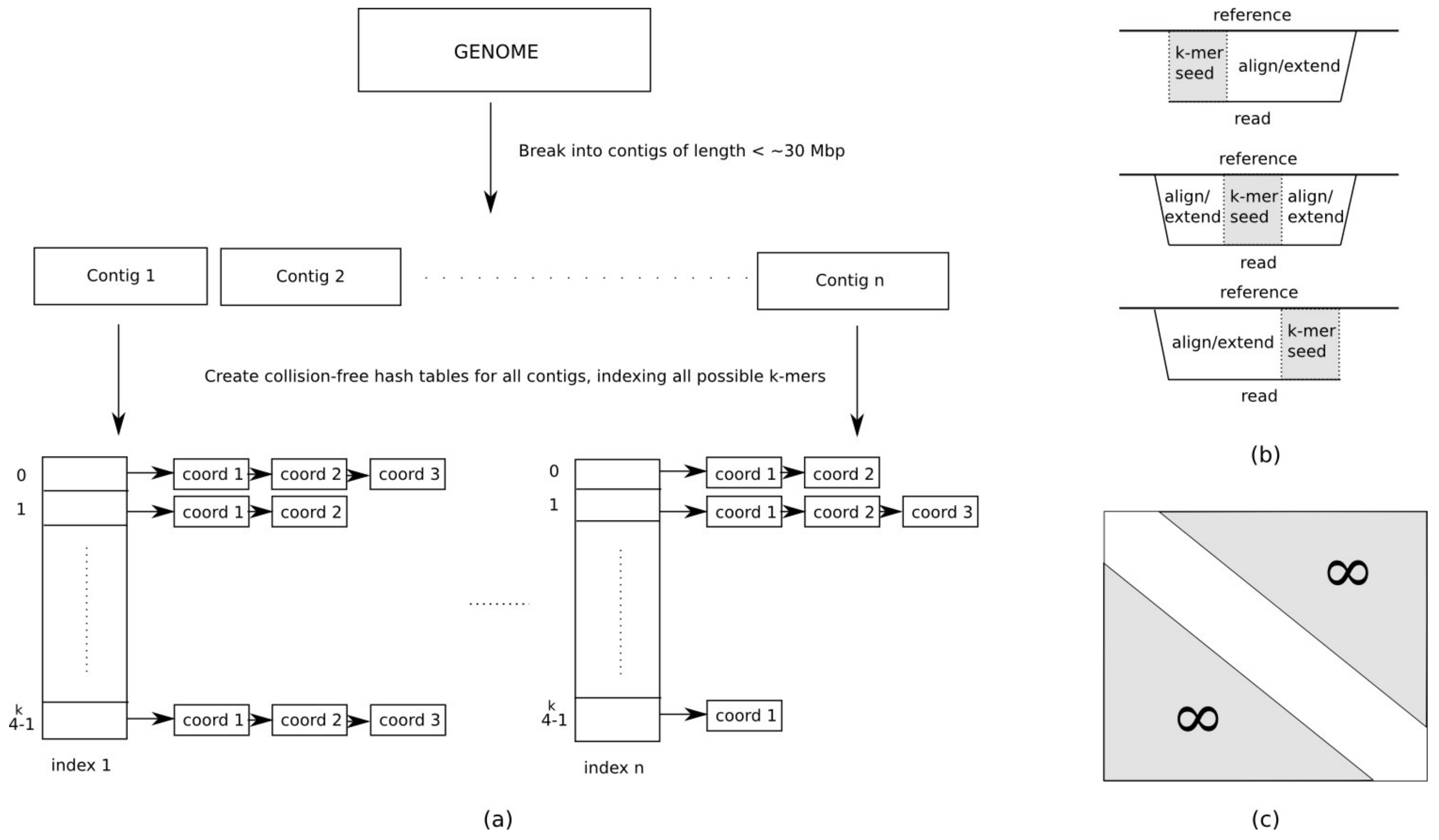
Short read mappers

- BWT-FM based
  - Illumina: BWA, Bowtie, SOAP2
  - Human genome can be compressed into a 2.3 GB data structure through BWT
  - Extremely fast for perfect hits
  - Increased memory lookups for mismatch
    - Indels are found in postprocessing when paired-end reads are available
  - GPGPU implementations: SOAP3 (poor performance due to memory lookups)

Read mappers: PacBio

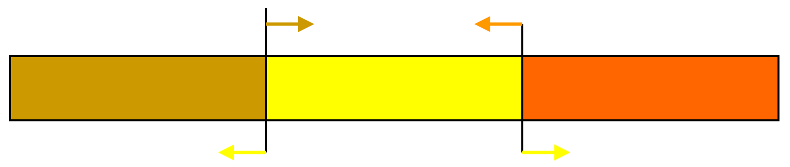
- BLASR aligner; tuned for PacBio error model (indel dominated, ~15%)
- Two versions:
  - Suffix array (hash) based
  - BWT-FM based

Hash Based Aligners



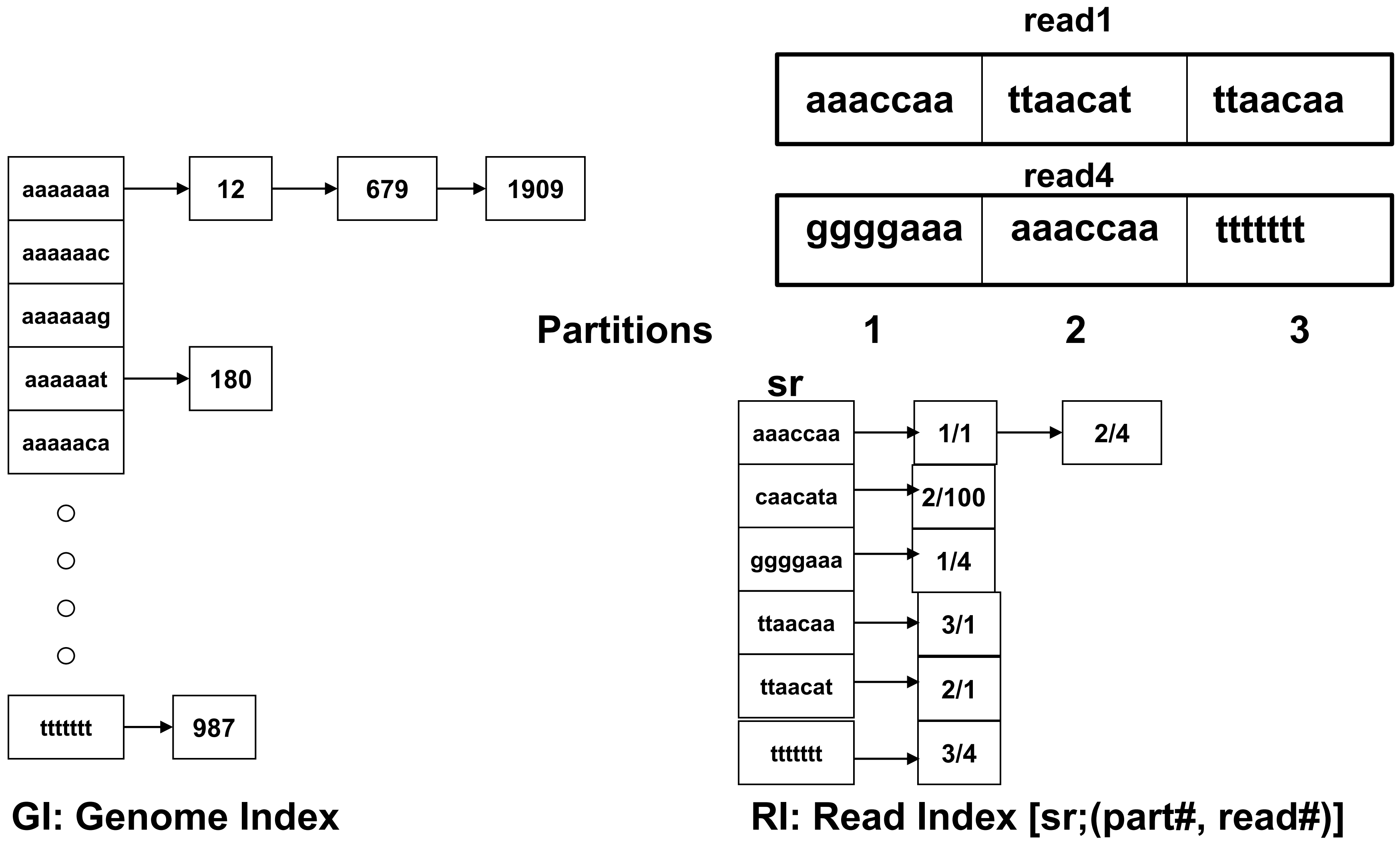
Seed and extend

- Break the read into *n* segments of k-mers.
  - For perfect sensitivity under edit distance *e*
    - There is at least one *l*-mer where l = floor(*L*/(e+1)); *L*=read length
    - For fixed *l*=*k*; *n* = *e*+1 and k ≤ *L* / *n*
  - Large k -> large memory
  - Small k -> more hash hits
- Lets consider the read length is 36 bp, and k=12.



- if we are looking for 2 edit distance (mismatch, indel) this would guaranty to find all of the hits

Cache oblivious search



GI: Genome Index

RI: Read Index [sr;(part#, read#)]

Cache oblivious search

- GI and RI are both sorted
- Scan GI; for all GI[i] = RI[j].*sr*
  - Map all partition/read_number combinations in RI[j]
  - All of the above have the same *sr* and its corresponding GI[i] list*;* therefore:
    - They have the same *seed* locations: same sequence content in the reference genome to *extend*
    - Once GI[i] and corresponding ref(GI[i].1, GI[i].2, …) are loaded from *main memory* to *cache memory;* then you re-use the **faster** cache memory contents; minimizing cache hits and main-to-cache transfers

Cache oblivious search

Mapper	Level 2 Cache Misses per Instruction	Instruction per cycle
Bowtie	0.0016	0.94
BWA	0.0016	0.93
MAQ	0.0060	0.56
mrsFAST	0.0008	1.24

Spaced seeds

- Instead of a k-mer with contiguous hit (1111..1); use space seeds
  - Seed S is defined by Length and Weight
- 0’s are “don’t care” characters
  - 111111001111111100 requires
    - 6 matches + 2 “don’t care”s + 8 matches + 2 “don’t care”s; a valid hit:

```
CGACTAGCTAGCTAGCTA
CGACTAAGTAGCTAGCGC
```

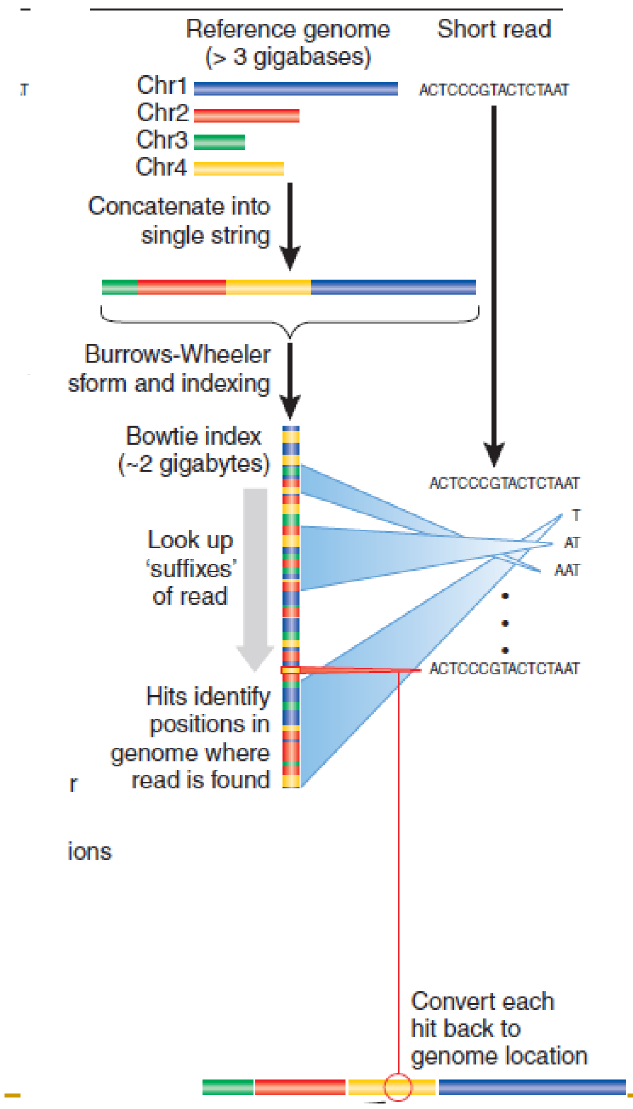
    - Length = 18; weight = 14

Spaced seeds

- You can define a set of N spaced seeds for read length R; and weight W that *guarantees* full sensitivity with less than *E* number of mismatches **without** the need for alignment step
  - **ZOOM!:** Zillions of oligos mapped
    - No dynamic programming for mismatch-only
    - Index the reads with N spaced seeds depending on R and W
    - Scan the reference genome in the read index

Lin et al. Bioinformatics, 2008

Burrows-Wheeler



- Store entire reference genome.
- Align tag base by base from the end.
- When tag is traversed, all active locations are reported.
- If no match is found, then back up and try a substitution.

Burrows-Wheeler Transformation

1. Append to the input string a special char, $, smaller than all alphabet.

mississippi$

Burrows-Wheeler Transformation (cnt'd)

2. Generate all rotations.

m	i	s	s	i	s	s	i	p	p	i	$
i	s	s	i	s	s	i	p	p	i	$	m
s	s	i	s	s	i	p	p	i	$	m	i
s	i	s	s	i	p	p	i	$	m	i	s
i	s	s	i	p	p	i	$	m	i	s	s
s	s	i	p	p	i	$	m	i	s	s	i
s	i	p	p	i	$	m	i	s	s	i	s
i	p	p	i	$	m	i	s	s	i	s	s
p	p	i	$	m	i	s	s	i	s	s	i
p	i	$	m	i	s	s	i	s	s	i	p
i	$	m	i	s	s	i	s	s	i	p	p
$	m	i	s	s	i	s	s	i	p	p	i

Burrows-Wheeler Transformation (cnt'd)

3. Sort rotations according to the alphabetical order.

$	m	i	s	s	i	s	s	i	p	p	i
i	$	m	i	s	s	i	s	s	i	p	p
i	p	p	i	$	m	i	s	s	i	s	s
i	s	s	i	p	p	i	$	m	i	s	s
i	s	s	i	s	s	i	p	p	i	$	m
m	i	s	s	i	s	s	i	p	p	i	$
p	i	$	m	i	s	s	i	s	s	i	p
p	p	i	$	m	i	s	s	i	s	s	i
s	i	p	p	i	$	m	i	s	s	i	s
s	i	s	s	i	p	p	i	$	m	i	s
s	s	i	p	p	i	$	m	i	s	s	i
s	s	i	s	s	i	p	p	i	$	m	i

Burrows-Wheeler Transformation (cnt'd)

4. Output the last column.

$	m	i	s	s	i	s	s	i	p	p	i
i	$	m	i	s	s	i	s	s	i	p	p
i	p	p	i	$	m	i	s	s	i	s	s
i	s	s	i	p	p	i	$	m	i	s	s
i	s	s	i	s	s	i	p	p	i	$	m
m	i	s	s	i	s	s	i	p	p	i	$
p	i	$	m	i	s	s	i	s	s	i	p
p	p	i	$	m	i	s	s	i	s	s	i
s	i	p	p	i	$	m	i	s	s	i	s
s	i	s	s	i	p	p	i	$	m	i	s
s	s	i	p	p	i	$	m	i	s	s	i
s	s	i	s	s	i	p	p	i	$	m	i

Burrows-Wheeler Transformation (cnt'd)

mississippi$

$$\downarrow$$

ipssm$pissii

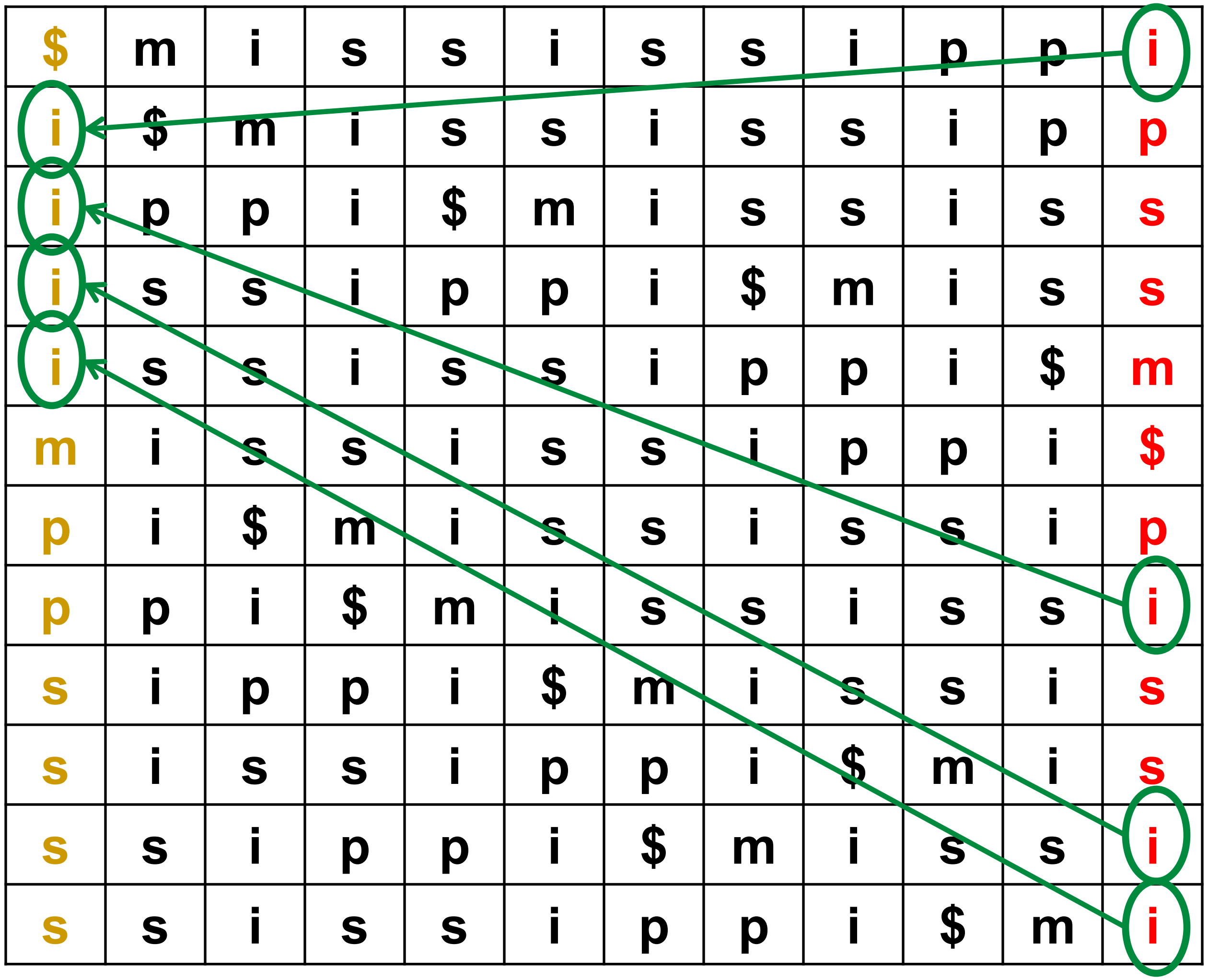
Ferragina-Manzini Index

First column: F

Last column: L

Let’s make an L to F map.

Observation: The nth i in L is the nth i in F.



$	m	i	s	s	i	s	s	i	p	p	i
i	$	m	i	s	s	i	s	s	i	p	p
i	p	p	i	$	m	i	s	s	i	s	s
i	s	s	i	p	p	i	$	m	i	s	s
i	s	s	i	s	s	i	p	p	i	$	m
m	i	s	s	i	s	s	i	p	p	i	$
p	i	$	m	i	s	s	i	s	s	i	p
p	p	i	$	m	i	s	s	i	s	s	i
s	i	p	p	i	$	m	i	s	s	i	s
s	i	s	s	i	p	p	i	$	m	i	s
s	s	i	p	p	i	$	m	i	s	s	i
s	s	i	s	s	i	p	p	i	$	m	i

Ferragina-Manzini Index: L to F map

Store/compute a two dimensional Occ(*j*,'c') table of the number of occurrences of char 'c' up to position *j* (inclusive).

and one dimensional Cnt('c') and Rank('c') tables

Occ(*j*,'c')

	$	i	m	p	s
i	0	1	0	0	0
p	0	1	0	1	0
s	0	1	0	1	1
s	0	1	0	1	2
m	0	1	1	1	2
$	1	1	1	1	2
p	1	1	1	2	2
i	1	2	1	2	2
s	1	2	1	2	3
s	1	2	1	2	4
i	1	3	1	2	4
i	1	4	1	2	4

Cnt('c')

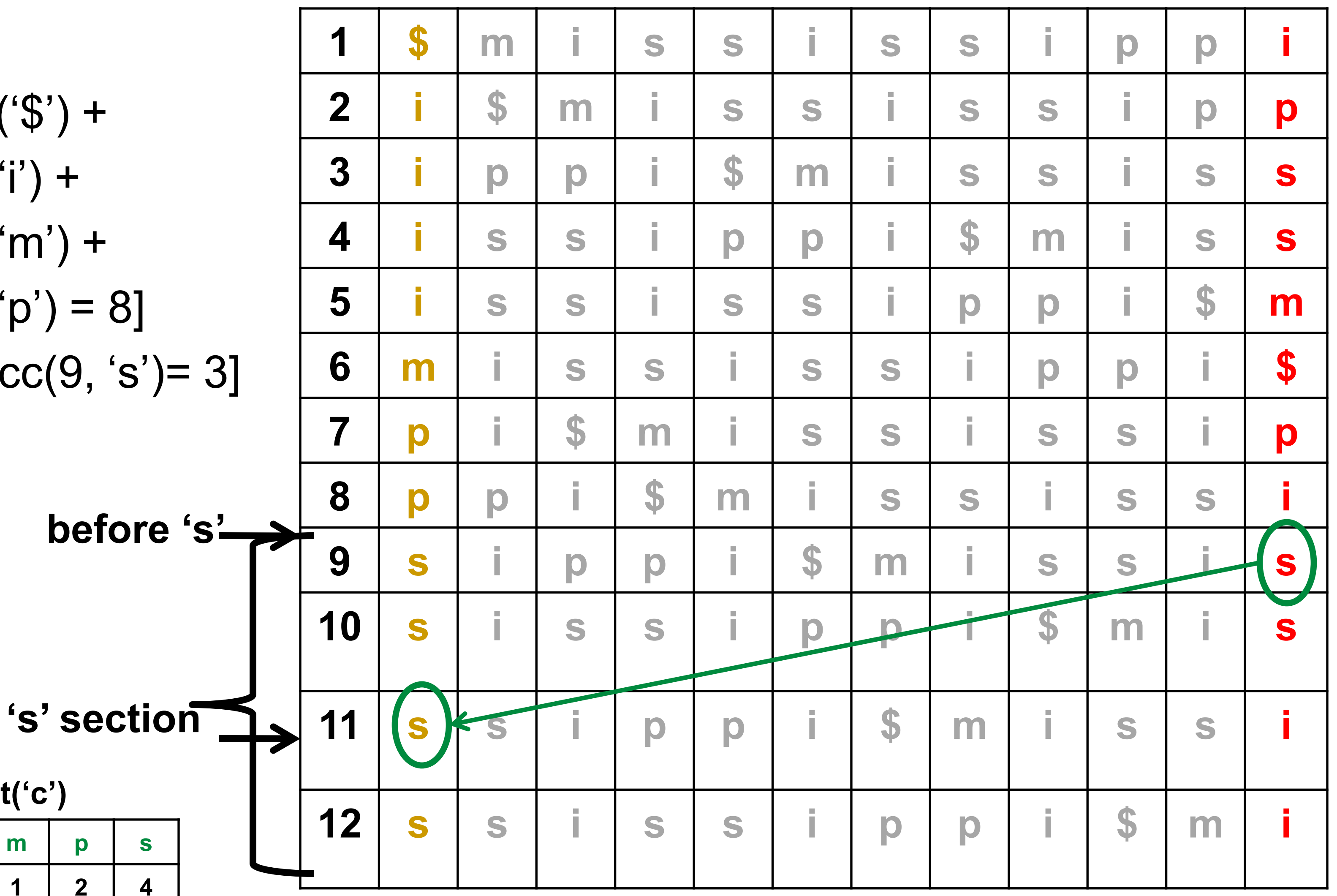
$	i	m	p	s
1	4	1	2	4

Rank('c')

$	i	m	p	s
12	2	1	9	3

Ferragina-Manzini Index: L to F map

[Cnt('$') +
Cnt('i') +
Cnt('m') +
Cnt('p') = 8]
+ [Occ(9, 's')= 3]
= 11



1	$	m	i	s	s	i	s	s	i	p	p	i
2	i	$	m	i	s	s	i	s	s	i	p	p
3	i	p	p	i	$	m	i	s	s	i	s	s
4	i	s	s	i	p	p	i	$	m	i	s	s
5	i	s	s	i	s	s	i	p	p	i	$	m
6	m	i	s	s	i	s	s	i	p	p	i	$
7	p	i	$	m	i	s	s	i	s	s	i	p
8	p	p	i	$	m	i	s	s	i	s	s	i
9	s	i	p	p	i	$	m	i	s	s	i	s
10	s	i	s	s	i	p	p	i	$	m	i	s
11	s	s	i	p	p	i	$	m	i	s	s	i
12	s	s	i	s	s	i	p	p	i	$	m	i

Cnt('c')

$	i	m	p	s
1	4	1	2	4

Ferragina-Manzini Index: Reverse traversal

(1) i
(2) p
(7) p
(8) i
(3) s
(9) s
(11) i
(4) s
(10) s
(12) i
(5) m
(6) $

1	$	m	i	s	s	i	s	s	i	p	p	i
2	i	$	m	i	s	s	i	s	s	i	p	p
3	i	p	p	i	$	m	i	s	s	i	s	s
4	i	s	s	i	p	p	i	$	m	i	s	s
5	i	s	s	i	s	s	i	p	p	i	$	m
6	m	i	s	s	i	s	s	i	p	p	i	$
7	p	i	$	m	i	s	s	i	s	s	i	p
8	p	p	i	$	m	i	s	s	i	s	s	i
9	s	i	p	p	i	$	m	i	s	s	i	s
10	s	i	s	s	i	p	p	i	$	m	i	s
11	s	s	i	p	p	i	$	m	i	s	s	i
12	s	s	i	s	s	i	p	p	i	$	m	i

Mapping with BWT-FM

Auxillary data structures for efficient pattern matching: how to find the corresponding chars in the first column efficiently, in terms of both time and space.

	a	c	g	T
rank	1	5	8	11

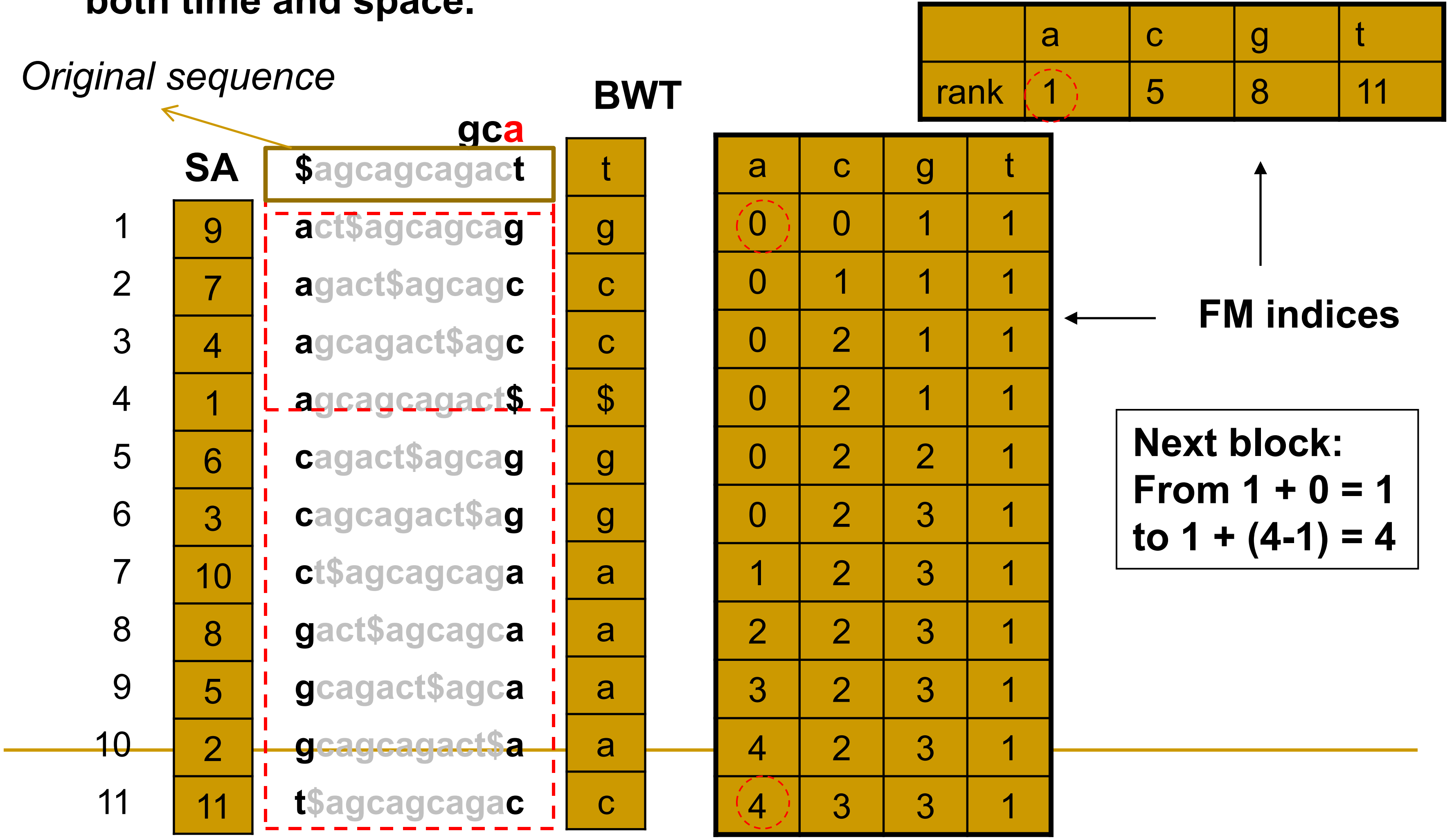
Original sequence

FM indices

	SA		**BWT**	a	c	g	t
		$agcagcagact	t				
1	9	act$agcagcag	g	0	0	1	1
2	7	agact$agcagc	c	0	1	1	1
3	4	agcagact$agc	c	0	2	1	1
4	1	agcagcagact$	$	0	2	1	1
5	6	cagact$agcag	g	0	2	2	1
6	3	cagcagact$ag	g	0	2	3	1
7	10	ct$agcagcaga	a	1	2	3	1
8	8	gact$agcagca	a	2	2	3	1
9	5	gcagact$agca	a	3	2	3	1
10	2	gcagcagact$a	a	4	2	3	1
11	11	t$agcagcagac	c	4	3	3	1

Mapping with BWT-FM

Auxillary data structures for efficient pattern matching: how to find the corresponding chars in the first column efficiently, in terms of both time and space.



	a	c	g	t
rank	1	5	8	11

	SA		BWT
		$agcagcagact	t
1	9	act$agcagcag	g
2	7	agact$agcagc	c
3	4	agcagact$agc	c
4	1	agcagcagact$	$
5	6	cagact$agcag	g
6	3	cagcagact$ag	g
7	10	ct$agcagcaga	a
8	8	gact$agcagca	a
9	5	gcagact$agca	a
10	2	gcagcagact$a	a
11	11	t$agcagcagac	c

a	c	g	t
0	0	1	1
0	1	1	1
0	2	1	1
0	2	1	1
0	2	2	1
0	2	3	1
1	2	3	1
2	2	3	1
3	2	3	1
4	2	3	1
4	3	3	1

Next block:
From 1 + 0 = 1
to 1 + (4-1) = 4

Mapping with BWT-FM

Auxillary data structures for efficient pattern matching: how to find the corresponding chars in the first column efficiently, in terms of both time and space.

	a	c	g	T
rank	1	5	8	11

Original sequence

gca

	SA		BWT	a	c	g	t
		$agcagcagact	t				
1	9	act$agcagcag	g	0	0	1	1
2	7	agact$agcagc	c	0	1	1	1
3	4	agcagact$agc	c	0	2	1	1
4	1	agcagcagact$	$	0	2	1	1
5	6	cagact$agcag	g	0	2	2	1
6	3	cagcagact$ag	g	0	2	3	1
7	10	ct$agcagcaga	a	1	2	3	1
8	8	gact$agcagca	a	2	2	3	1
9	5	gcagact$agca	a	3	2	3	1
10	2	gcagcagact$a	a	4	2	3	1
11	11	t$agcagcagac	c	4	3	3	1

FM indices

Next block:
From 5 + 0 = 5
to 5 + (2-1) = 6

Mapping with BWT-FM

Auxillary data structures for efficient pattern matching: how to find the corresponding chars in the first column efficiently, in terms of both time and space.

Original sequence

gca

	SA		BWT
		$agcagcagac**t**	t
1	9	**a**ct$agcagca**g**	g
2	7	**a**gact$agcag**c**	c
3	4	**a**gcagact$ag**c**	c
4	1	**a**gcagcagact**$**	$
5	6	**c**agact$agca**g**	g
6	3	**c**agcagact$a**g**	g
7	10	**c**t$agcagcag**a**	a
8	8	**g**act$agcagc**a**	a
9	5	**g**cagact$agc**a**	a
10	2	**g**cagcagact$**a**	a
11	11	**t**$agcagcaga**c**	c

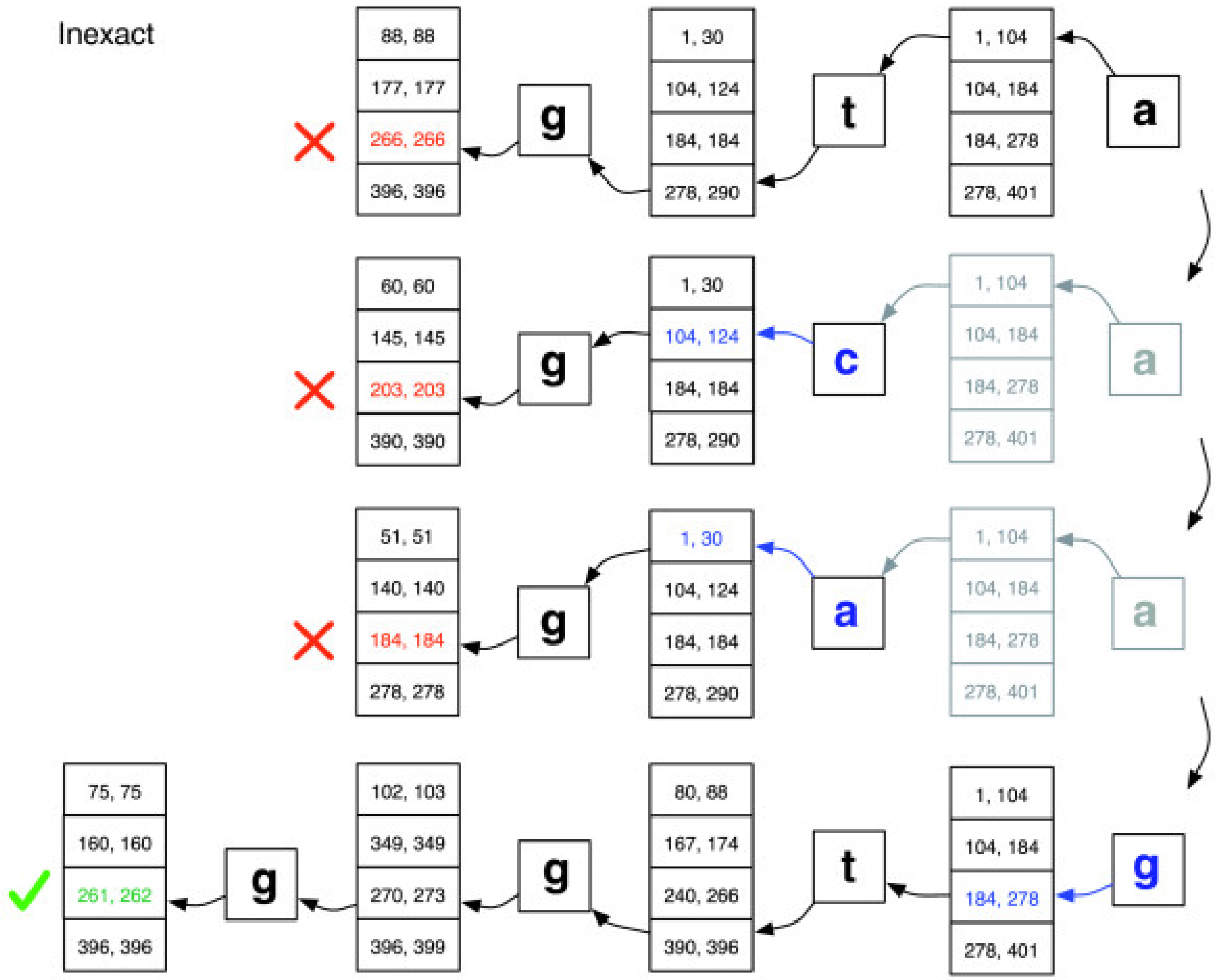
	a	c	g	T
rank	1	5	8	11

FM indices

a	c	g	t
0	0	1	1
0	1	1	1
0	2	1	1
0	2	1	1
0	2	2	1
0	2	3	1
1	2	3	1
2	2	3	1
3	2	3	1
4	2	3	1
4	3	3	1

Next block:
From 8 + 1 = 9
to 8 + (3-1) = 10

Inexact match



Mapping Quality

- MAPQ = -10 * $\log_{10}$(Prob(mapping is wrong))

For reference sequence *x;* read sequence *z:*
***p(z | x,u)* =** probability that *z* comes from position *u*
= multiplication of p_e of mismatched bases of *z*

For posterior probability **p(u | x,z)** assume uniform prior distribution **p(u|x)**
L=|x| and *l*=|z|. Apply Bayesian formula:

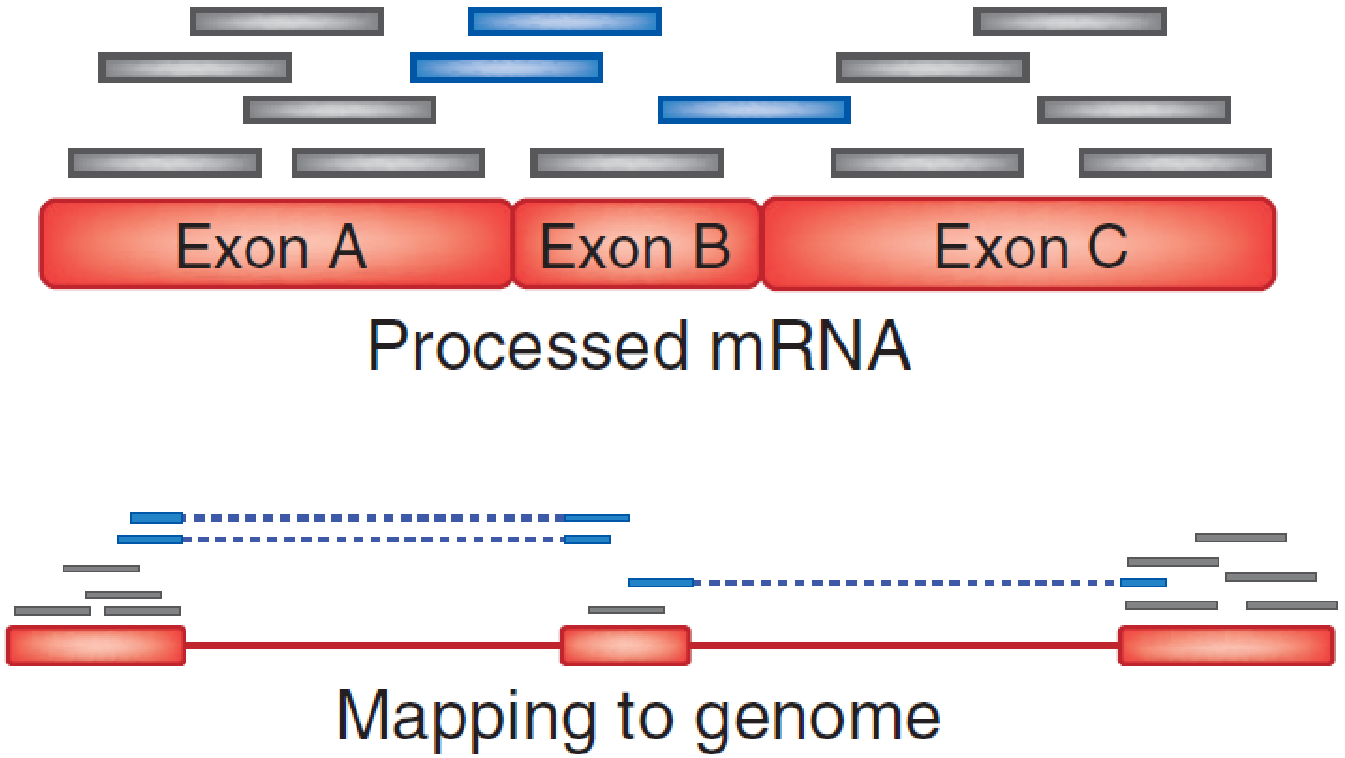
$$p_s(u|x,z) = \frac{p(z|x,u)}{\sum_{v=1}^{L-l+1} p(z|x,v)}$$

$$Q_s(u|x,z) = -10 \log_{10}[1 - p_s(u|x,z)].$$

Calculated for one "best" hit

Li et al., Genome Research, 2008

Spliced-read mapping



- Used for processed mRNA data
- Reports reads that span introns.
- Examples: TopHat, ERANGE